

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 14 FEB. 2003

WIPO PCT

10/031,216

Applicant's or agent's file reference PCT/98-22	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US01/11825	International filing date (day/month/year) 10 April 2001 (10.04.2001)	Priority date (day/month/year) 10 April 2000 (10.04.2000)	
International Patent Classification (IPC) or national classification and IPC IPC(7): C12N 1/38, 1/22; C12P 7/56, 7/10 and US Cl.: 435/161, 163, 165			
Applicant MIDWEST RESEARCH INSTITUTE			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 0 sheets.

RECEIVED

3. This report contains indications relating to the following items:

- | | | | |
|------|-------------------------------------|---|---|
| I | <input checked="" type="checkbox"/> | Basis of the report | <p>MAY 19 2003</p> <p>TECH CENTER 1600/2900</p> |
| II | <input type="checkbox"/> | Priority | |
| III | <input type="checkbox"/> | Non-establishment of report with regard to novelty, inventive step and industrial applicability | |
| IV | <input type="checkbox"/> | Lack of unity of invention | |
| V | <input checked="" type="checkbox"/> | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement | |
| VI | <input type="checkbox"/> | Certain documents cited | |
| VII | <input type="checkbox"/> | Certain defects in the international application | |
| VIII | <input type="checkbox"/> | Certain observations on the international application | |

Date of submission of the demand 02 November 2001 (02.11.2001)	Date of completion of this report 11 September 2002 (11.09.2002)
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Jon P. Weber, Ph.D. <i>Janice Ford</i> Telephone No. 703-308-0196

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US01/11825

I. Basis of the report**1. With regard to the elements of the international application:***

- ☒ the international application as originally filed.
- ☒ the description:
pages 1-9 as originally filed
pages NONE filed with the demand
pages NONE filed with the letter of _____.
- ☒ the claims:
pages 10 and 11 as originally filed
pages NONE as amended (together with any statement) under Article 19
pages NONE filed with the demand
pages NONE filed with the letter of _____.
- ☒ the drawings:
pages 1-2 as originally filed
pages NONE filed with the demand
pages NONE filed with the letter of _____.
- ☐ the sequence listing part of the description:
pages NONE as originally filed
pages NONE filed with the demand
pages NONE filed with the letter of _____.

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages NONE
- ☐ the claims, Nos. NONE
- ☐ the drawings, sheets/fig NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US01/11825

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 6-9	YES
	Claims 1-5 AND 10	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-10	NO
Industrial Applicability (IA)	Claims 1-10	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-5 and 10 lack novelty under PCT Article 33(2) as being anticipated by Perego et al. (1994).

Perego et al. (1994) disclose fermenting hardwood hydrolysates by *Pachysolen tannophilus* after the hydrolysates have been treated with fly ash at acidic pH, boiling 1 hour, and filtered to remove solids. Fly ash is reported to contain 54.41% SiO₂, 23.25% Al₂O₃, 5.92% Fe₂O₃, 1.35% TiO₂ and other metal oxides (Table 2). Additional nutrients are added before fermentation.

Claims 1-10 lack an inventive step under PCT Article 33(3) as being obvious over Perego et al. (1994) in view of Parajó et al. (1997).

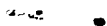
The teachings of Perego et al. (1994) have been discussed above. Perego et al. (1994) lack the fermenting organisms of claim 6, the amount of metal oxide as titanium dioxide, and using softwood hydrolysate.

Parajó et al. (1997) disclose that lignocellulosic biomass is widely used as a source of fermentable material that can be converted to fuels by suitable organisms such as *Candida*. The wood specifically used was *Eucalyptus globulus* a well-known softwood tree.

A person of ordinary skill in the art would have been motivated to use yeasts to ferment softwood hydrolysates by the method of Perego et al. (1994) because Parajó et al. (1997) establish that yeasts are commonly used to ferment softwood lignocellulosic biomass into fuels. The adjustment of the amount of a particular metal oxide, such as titanium oxide, is within the skill of the ordinary artisan to optimise. Perego et al. (1994) used fly ash as a source of a mixture of metal oxides comprising, *inter alia*, titanium oxide because of its low cost and ready availability. Hence it would be *prima facie* obvious to optimise the amount of titanium oxide, or to use yeasts to ferment softwood hydrolysates.

Claims 1-10 meet the criteria for industrial applicability set out in PCT Article 33(4), because the process converts waste biomass into useful fuels.

----- NEW CITATIONS -----□



PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 12 April 2002 (12.04.02)	
International application No. PCT/US01/11825	Applicant's or agent's file reference PCT/98-22
International filing date (day/month/year) 10 April 2001 (10.04.01)	Priority date (day/month/year) 10 April 2000 (10.04.00)
Applicant HAMES, Bonnie, R. et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

02 November 2001 (02.11.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Odile ALIU Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PCT/98-22	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 01/ 11825	International filing date (day/month/year) 10/04/2001	(Earliest) Priority Date (day/month/year) 10/04/2000
Applicant MIDWEST RESEARCH INSTITUTE et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

METHOD FOR THE SELECTIVE REMOVAL OF FERMENTATION INHIBITORS FROM BIOMASS HYDROLYZATE

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1
☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

T/US 01/11825

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 7 C12N1/38 C12N1/22 C12P7/56 C12P7/10		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N C12P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data, PAJ		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PEREGO P ET AL: "Acid hemicellulose hydrolysate: Physical treatments and continuous immobilized-cell fermentations." BIOPROCESS ENGINEERING, vol. 10, no. 1, 1994, pages 35-41, XP001016151 ISSN: 0178-515X the whole document ---	1,3,10
X	US 3 998 732 A (SOLBACH HERMANN ET AL) 21 December 1976 (1976-12-21) the whole document --- -/--	1,3,10
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents :		
A document defining the general state of the art which is not considered to be of particular relevance		
E earlier document but published on or after the international filing date		
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		
T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention		
X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone		
Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.		
* & * document member of the same patent family		
Date of the actual completion of the international search 26 September 2001		Date of mailing of the international search report 04/10/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Lejeune, R



“ ” “ ”

INTERNATIONAL SEARCH REPORT

International Application No

T/US 01/11825

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category ~	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>PARAJO J C ET AL: "Improved xylitol production with <i>Debaryomyces hansenii</i> Y-7426 from raw or detoxified wood hydrolysates."</p> <p>ENZYME AND MICROBIAL TECHNOLOGY, vol. 21, no. 1, 1997, pages 18-24, XP001016128 ISSN: 0141-0229 the whole document</p> <p>---</p>	1-10
A	<p>MACHADO A E H ET AL: "PHOTOCATALYTIC DEGRADATION OF LIGNIN AND LIGNIN MODELS, USING TITANIUM DIOXIDE: THE ROLE OF THE HYDROXYL RADICAL"</p> <p>CHEMOSPHERE, PERGAMON PRESS, OXFORD, GB, vol. 40, no. 1, 2000, pages 115-124, XP001024191 ISSN: 0045-6535 the whole document</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

T/US 01/11825

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 3998732	A	21-12-1976	DE 2449756 A1	22-04-1976
			AT 343561 B	12-06-1978
			AT 799375 A	15-09-1977
			BR 7506827 A	17-08-1976
			CA 1047411 A1	30-01-1979
			CH 619909 A5	31-10-1980
			DD 121922 A5	05-09-1976
			ES 441846 A1	01-04-1977
			FI 752846 A ,B,	20-04-1976
			FR 2288062 A1	14-05-1976
			IT 1060803 B	30-09-1982
			JP 1186245 C	20-01-1984
			JP 51064759 A	04-06-1976
			JP 58012074 B	05-03-1983
			NO 753510 A ,B,	21-04-1976
			SE 409573 B	27-08-1979
			SE 7511318 A	20-04-1976

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 October 2001 (18.10.2001)

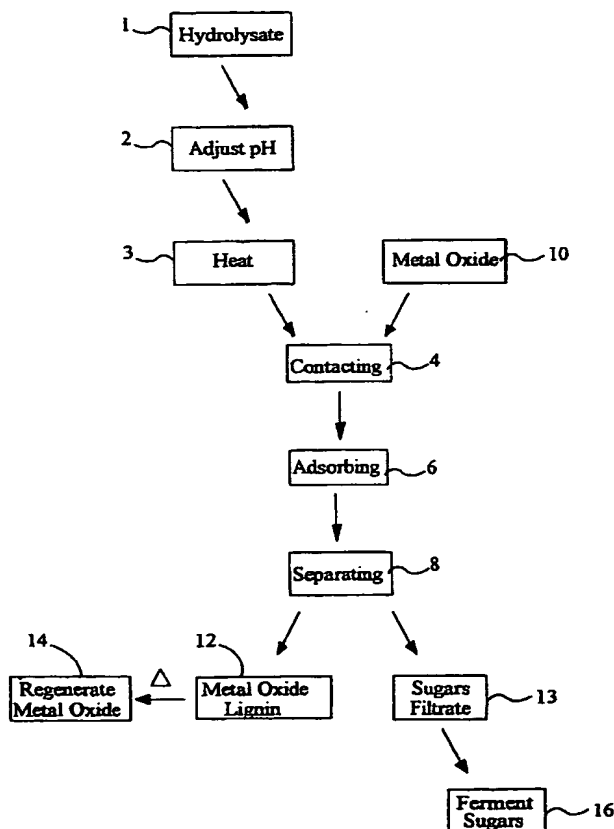
PCT

(10) International Publication Number
WO 01/77296 A1

- (51) International Patent Classification⁷: C12N 1/38, 1/22, C12P 7/56, 7/10
- (21) International Application Number: PCT/US01/11825
- (22) International Filing Date: 10 April 2001 (10.04.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/195,416 10 April 2000 (10.04.2000) US
- (71) Applicant (for all designated States except US): MID-WEST RESEARCH INSTITUTE [US/US]; 425 Volker Boulevard, Kansas City, MO 64110 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): HAMES, Bonnie, R. [US/US]; 2522 West 104th Circle, Westminster, CO 80234 (US). HAVERCAMP, Amie, D. [US/US]; 7747 West 51st Street, Arvada, CO 80002 (US). HAYWARD, Tammy, K. [US/US]; 1240 West 11th Court, Broomfield, CO 80020 (US). NAGLE, Nicholas, J. [US/US]; 3156 West 11th Avenue Court, Broomfield, CO 80020 (US).
- (74) Agent: WHITE, Paul, J.; National Renewable Energy Laboratory, 1617 Cole Boulevard, Golden, CO 80401 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,

[Continued on next page]

(54) Title: METHOD FOR THE SELECTIVE REMOVAL OF FERMENTATION INHIBITORS FROM BIOMASS HYDROLYZATE



(57) Abstract: A process of making a fuel or chemical from a biomass hydrolyzate is provided which comprises the steps of providing a biomass hydrolyzate, adjusting the pH of the hydrolyzate, contacting a metal oxide having an affinity for guaiacyl or syringyl functional groups, or both and the hydrolyzate for a time sufficient to form an adsorption complex; removing the complex wherein a sugar fraction is provided, and converting the sugar fraction to fuels or chemicals using a microorganism.

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MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL,
TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(48) Date of publication of this corrected version:

3 January 2002

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(15) Information about Correction:

see PCT Gazette No. 01/2002 of 3 January 2002, Section II

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 October 2001 (18.10.2001)

PCT

(10) International Publication Number
WO 01/77296 A1

(51) International Patent Classification⁷: **C12N 1/38**,
1/22, C12P 7/56, 7/10

(21) International Application Number: PCT/US01/11825

(22) International Filing Date: 10 April 2001 (10.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/195,416 10 April 2000 (10.04.2000) US

(71) Applicant (for all designated States except US): **MID-
WEST RESEARCH INSTITUTE** [US/US]; 425 Volker
Boulevard, Kansas City, MO 64110 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HAMES, Bonnie, R.**

[US/US]; 2522 West 104th Circle, Westminster, CO 80234
(US). **HAVERCAMP, Amie, D.** [US/US]; 7747 West 51st
Street, Arvada, CO 80002 (US). **HAYWARD, Tammy, K.**
[US/US]; 1240 West 11th Court, Broomfield, CO 80020
(US). **NAGLE, Nicholas, J.** [US/US]; 3156 West 11th Av-
enue Court, Broomfield, CO 80020 (US).

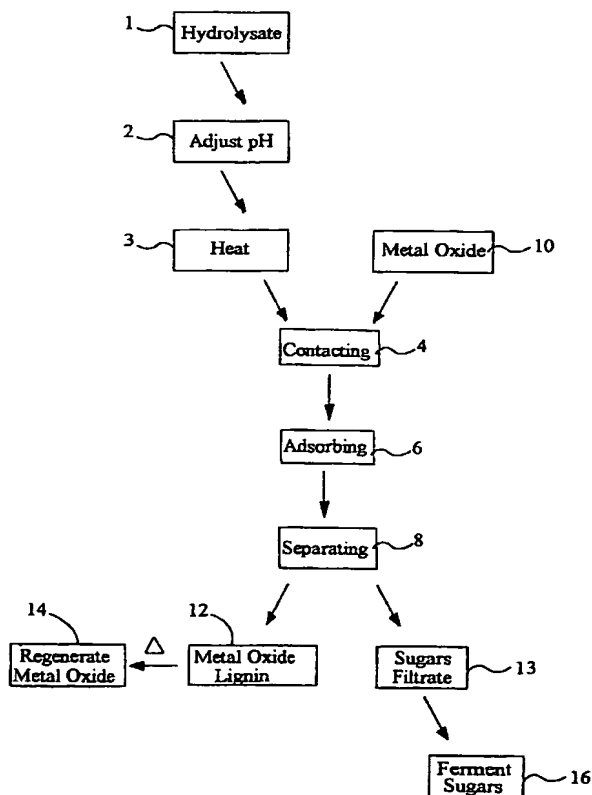
(74) Agent: **WHITE, Paul, J.**; National Renewable Energy
Laboratory, 1617 Cole Boulevard, Golden, CO 80401 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL,
TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European

[Continued on next page]

(54) Title: IMPROVED PROCESS FOR THE CONVERSION OF AN AQUEOUS BIOMASS HYDROLYZATE INTO FUELS
OR CHEMICALS BY THE SELECTIVE REMOVAL OF FERMENTATION INHIBITORS



(57) Abstract: A process of making a fuel or chemical from a biomass hydrolyzate is provided which comprises the steps of providing a biomass hydrolyzate, adjusting the pH of the hydrolyzate, contacting a metal oxide having an affinity for guaiacyl or syringyl functional groups, or both and the hydrolyzate for a time sufficient to form an adsorption complex; removing the complex wherein a sugar fraction is provided, and converting the sugar fraction to fuels or chemicals using a microorganism.

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patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

Published:

— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

**METHOD FOR THE SELECTIVE REMOVAL OF
FERMENTATION INHIBITORS FROM BIOMASS HYDROLYZATE**

5 Contractual Origin of the Invention:

The United States Government has rights in this invention pursuant to Contract No. DE-AC36-99GO10337 between the United States Department of Energy and the Midwest Research Institute.

Technical Field:

10 This invention relates to industrial fuels and chemicals, and in particular to an improved process for the removal of lignin-derived phenolic compounds from dissolved sugars in an aqueous biomass hydrolyzate and the biological conversion of the sugars into fuels and chemicals.

Background Art:

15 As is well known in the biological conversion art, the traditional method for detoxifying a biomass hydrolyzate liquor is overliming. Overliming has been widely used since the 1940's. Leonard, R.H. and Hajny, G.J. Fermentation of Wood Sugars to Ethyl Alcohol, *Industrial and Engineering Chemistry*, vol.37, No. 4, p.p. 390-395 (1945). The basic steps in the overliming process include: adjusting the pH of the hydrolyzate to 10.0 ± 0.1 using $\text{Ca}(\text{OH})_2$ or lime,
20 heating the hydrolyzate to 60°C for 30 minutes, filtering the heated hydrolyzate to remove precipitated solids, and acidifying the filtrate to a pH optimum, which is efficient for the bio-conversion of the dissolved sugars into the desired product(s).

A distinct disadvantage of the overliming process is the difficulty in controlling the pH adjustment step. Where a fermentable substrate is a dissolved sugar, strict control over the pH
25 adjustment step is critical because, at a pH greater than 10 one experiences a degradation in the fermentable carbohydrate fraction. It is a further disadvantage in that the mechanism of overliming is not well understood, thereby making it difficult to optimize the process. Moreover, overliming does not detoxify hydrolysis liquors to the extent that an undiluted hydrolyzate comprises an efficient fermentable substrate (overliming allows fermentation at
30 30%-50% liquor concentration). It is also desirable, in some overliming applications, to use less

lime, thereby resulting in the production of less insoluble gypsum, which has been found to precipitate in process lines.

Disclosure of the Invention:

5 Therefore, it is an object of the invention to provide a process for converting a biomass hydrolyzate into fuels or chemicals using a microbial culture.

 It is a further object of the invention to provide a process for detoxifying a biomass hydrolyzate for use as a conversion substrate.

10 It is still a further object of the invention to provide a process of converting a hydrolysis liquor without first diluting the liquor as a component of the medium.

 It is yet another object of the invention to improve the product yield in a microbial chemostat process for converting a biomass hydrolyzate into fuels and chemicals, such as ethanol or lactic acid.

15 Briefly, the invention provides a process of making a fuel or chemical from a biomass hydrolyzate comprising the steps of providing a biomass hydrolyzate, adjusting pH of the hydrolyzate, contacting a metal oxide having an affinity for guaiacyl or syringyl functional groups or both, and the hydrolyzate for a time sufficient to form an adsorption complex; removing the complex wherein a fermentable sugar fraction is provided, and converting the sugar fraction into a fuel or chemical using a microorganism.

20 The foregoing specific objects and advantages of the invention are illustrative of those which can be achieved by the present invention and are not intended to be exhaustive or limiting of the possible advantages which can be realized. Thus, those and other objects and advantages of the invention will be apparent from the description herein or can be learned from practicing the invention, both as embodied herein or as modified in view of any variations which may be
25 apparent to those skilled in the art.

Brief Description of the Drawings:

 The accompanying drawings, which are incorporated in and which constitute a part of the specification, illustrate at least one embodiment of the invention and, together with the description, explains the principles of the invention.

30 Figure 1 is a flow chart of an embodiment of the process.

Figure 2 is a graphical illustration that demonstrates the affinity for oxygen molecules adjacent to the lignin aromatic ring for a titanium dioxide surface.

Description of Preferred Embodiments:

Unless specifically defined otherwise, all technical or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

As used herein the term "phenolic" is an adjective meaning a member of the class of phenols. "Phenols" means the class of aromatic compounds in which one or more hydroxyl groups are attached directly to a benzene ring. Examples of phenols include phenol, cresol and resorcinol. "Biomass or lignin-derived phenols" include the compounds known as guaiacol, syringol, isoeugenol and vanillin.

A microbial process of converting a biomass hydrolyzate, such as wood, into fuels and chemicals is described. The process uses adsorption of the biomass hydrolyzate on a solid metal oxide support, such as titanium dioxide, for the selective removal of substances, such as lignin-derived compounds, which inhibit product formation in a traditional chemostat culture. The adsorption step is highly selective, to provide an efficient method for the fractionation of the hydrolyzate, 90% of the hydrolyzate's dissolved lignin-derived compounds being removed without a measurable decrease in the hydrolyzate's dissolved sugar concentration. Dissolved sugars and lignin-derived phenolic compounds are thereby fractionated into a fermentable medium for use in a traditional microbial chemostat process for making fuel and chemical products. The invention improves the product yield over the prior art methods for making fuels and chemicals from an undiluted biomass substrate.

With reference now to the drawing figures, Figure 1 is a flow chart of a preferred embodiment of the process according to the invention herein. In Figure 1, a biomass hydrolyzate liquor 1 is preferably obtained from a sulfuric acid hydrolyzed soft or hardwood. The pH of hydrolyzate is about 2.0. The hydrolyzate liquor 1 is adjusted to a pH within the range of 6-10, preferably 8.0-9.2, using $\text{Ca}(\text{OH})_2$. The removal of reactants from the hydrolysis liquor 1 which

inhibit product formation increases with an upward adjustment in pH to 10, which is the pH where the fermentable carbohydrate fraction begins to degrade.

After the adjust pH or adjusting 2 step of the pH of the hydrolyzate liquor 1 it, may but need not, be heated 3 to a temperature in the range of 60°C-90°C, preferably 90°C, and the
5 heated hydrolysis liquor is mixed in suspension for contacting with a metal oxide 10, such as a Norton Chemical Process Products Company, Akron, Ohio, high-surface-area TiO₂ 1/8" extrudate, type XT 25384, or anatase TiO₂, Aldrich Chemical Company. The suspension is allowed to cool at room temperature. High separation efficiency is generally achieved by using an amount (wt / wt) of TiO₂ 10 which is twice the estimated phenol concentration of the
10 hydrolysis liquor 1. This ratio varies, however, with the form, source, active surface area and liquid-contact surface area of the TiO₂ to be used. For example, where a softwood is the starting material for the hydrolyzate, absorption requires a 4:1 oxide to lignin-derived compounds weight ratio in order to sufficiently remove those substances from the liquor which inhibit product formation. Separation efficiency also varies with the age of the aqueous mixture and the source
15 of the biomass hydrolyzate 1. Producing hydrolyzate under conditions of high severity will also reduce the separation efficiency for removing inhibitory reactants.

Selective adsorbing step 6 is accomplished using all grades of TiO₂. Rate and adsorption efficiency of TiO₂ for aromatic compounds depends on the area of active TiO₂ surface area. Anatase titanium dioxide is preferred over the rutile form, and may comprise any high-surface-
20 area formulation, such as powders, thin-films, sol-gel crystals, or extruded pellets. Vanadium and zirconium oxides and, when processing at a pH in a range of greater than 7.0, manganese dioxide, silica, and alumina, may also be used as an adsorbent.

A batch reaction vessel or plug-flow reactor may be used as an adsorption vessel, depending upon the quantity of the aromatic compounds to be separated from the hydrolyzate
25 prior to the fermentation step. In the batch reactor a retention time of 30 min. is preferred. In a plug-flow reactor the preferred retention time is 15 minutes. Adsorption retention for these lengths of time typically results in a removal of up to 90 weight percent (wt %) of the aromatic compounds from the hydrolyzate 1 that inhibit product formation. Longer retention times are

desirable where the adsorption step 6 is processed at a low pH. The adsorption is slow in the pH range of 2 - 6 and more rapid when the pH is in the range of 7-10.

The adsorbing step 6 retains its efficiency and selectivity throughout a temperature in the range of 20°C-90°C. Also conditions of elevated temperature and pH do not affect adsorption selectivity relative to the soluble carbohydrate fraction. However, above pH 6 some carbohydrate degradation products, such as 5-(hydroxymethyl) furfural (HMF) and furfural, are adsorbed on the metal oxide 10 surface. Furthermore, under basic conditions at high temperature some entrainment of soluble salts occurs on the metal oxide surface when the mixture includes a salt, having low solubility at high temperature, such as calcium sulfate.

Increasing both the temperature and pH does not affect adsorption selectivity for lignin-derived compounds.

After contacting 4 the hydrolyzate 1 and metal oxide 2 adsorption preferably includes agitating the suspension for a time sufficient to allow colloidal particles to deposit on the TiO₂ surface. A clearing of the suspension when it is allowed to settle at room temperature for 1 hour is indicative of the adsorbed complex having been formed. The adsorbed complex is then separated in separation step 8, such as with a glass-fiber filter. The resulting sugars filtrate 13 thereby includes the fermentable carbohydrate fraction useful for conversion into fuels or chemicals. The filtrate 13, of the fractionated hydrolyzate liquor, may be used as an undiluted carbohydrate source for conversion into fuels and chemicals, by any well known microbial process, including ethanol or lactic acid.

Referring now to Figure 2, one unique aspect of the adsorption step 6 is its high separation selectivity of the metal oxide for lignin-derived compounds present in an aqueous hydrolysis liquor having an excess of monomer fermentable sugars. Greater than 90% of the soluble lignin component is removed from the hydrolyzate without any loss of glucose or xylose.

When using TiO₂ extruded pellets as an adsorbent, a slight concentration effect is observed during the adsorption process. This effect may be due to hydration of the metal oxide and the exclusion of sugars from the metal oxide's surface. Solid state nuclear magnetic resonance (nmr) analysis of lignin-derived model compounds, adsorbed on a TiO₂ surface, shows that lignin-derived selectivity is due, at least in part, to the affinity of titanium for adjacent oxygen molecules which are located on the aromatic ring structure of the adsorbed substrates. See

Figure 2, Guaiacol: X=Y=H; Isoeugenol: X = CH₃CH=CH-, Y=H; Syringol: X=H, Y=OMe.

Through chemical shifts in ¹³C nmr between lignin model compounds, both before and after formation of the complex with titanium alkoxides, it has been found that these molecules bind preferentially through the phenol oxygen and the oxygen of the adjacent methoxyl group. The

5 biomass-derived aromatic substituents have two main substitution patterns, commonly known as guaiacyl and syringyl, which contain the functional groups necessary for selective fractionation.

Although titanium, and many other transition metals, is known to be highly oxyphilic the affinity of this functionality for these metal oxides is so strong that in the presence of these aromatic compounds even highly oxygenated carbohydrate-derived compounds are excluded from the
10 metal oxide surface.

The process may, but need not, include a regeneration step 14. In this step, metal oxide adsorbents are regenerated using combustion of the adsorbed complex or metal oxide lignin 12 for 15 minutes at 400°C. An estimated 100 to 500 regeneration cycles may be utilized without a significant reduction in lignin-derived compound selectivity. Regeneration 14 of TiO₂ at a
15 temperature of less than 600°C avoids an anatase to rutile form conversion, which decreases the metal oxide's capacity for adsorption. A simple regeneration wash step using dilute sulfuric acid may also be used to increase the lifetime of TiO₂ adsorbents when the contacting step 4 is carried out at a pH greater than 7. When manganese dioxide is used, the adsorbent regeneration 14 has also been demonstrated for 15 minutes at 575°C.

20 EXAMPLE 1

This example demonstrates the resulting lignin-derived compound and glucose concentrations after adsorption of a biomass hydrolyzate using three different sets of process variables. Sample No. 1 was prepared using a 50 mL aliquot of a well mixed hydrolysis liquor, containing a large amount of suspended solids, and 10 grams of Norton high-surface-area TiO₂,
25 in a 100 mL beaker. The sample was agitated by hand for approximately 5 minutes until the mixture became clear (colloidal particulates deposited on the surface of the TiO₂). The mixture was allowed to settle at room temperature for 1 hour and filtered through a glass filter, Watman GFC, the filtrate being stored in a glass container.

Samples Nos. 2 and 3 were prepared by mixing a 50 mL aliquot of a well-mixed hydrolysis liquor, containing a large amount of suspended solids, in contact with 10 grams of TiO₂, high surface area, in a 100 ml beaker. Unlike sample No. 1, the TiO₂ in sample No. 2 was left in solution at room temperature without stirring for 8 hours (sample No. 2) prior to the separation of the TiO₂ adsorption complex by gravity filtration. Half of the liquid (approximately 20 ml), of sample No. 2, was decanted and filtered for lignin-derived compound and glucose determination, and the other half was retained in contact with TiO₂ overnight (sample No. 3). The beaker was covered with aluminum foil to minimize evaporation. After 20 hours, the remaining liquid of sample No. 3 was decanted from the TiO₂ adsorption complex and filtered through a glass Watman GFC filter. Filtrates for each sample were diluted with dilute H₂SO₄ and analyzed for their phenol concentrations by measuring UV absorbency at 204 nm. In addition, glucose concentrations for the filtrates were measured using a YSI, Yellow Springs Instruments Co., glucose analyzer.

The results of this example are summarized in Table 1. In the table, most of the adsorption occurs within the first hour and very little change is observed in either adsorption or selectivity with prolonged exposure. The absorbency at 204 nm (measured using a UV/visible spectrophotometer) reflects the concentration of the phenolic compounds remaining in the treated liquor. The absorbency at 282 nm reflects the concentration of furfural and 5-(hydroxymethyl) furfural.

TABLE 1

Sample	Absorbance 204 nm	Absorbance 282 nm	Lignin mg/mL	Glucose mg/mL
Original Liquor	1.0763	0.591308	6.3	9.4
Sample No. 1 (1 h)	2.14941	0.409973	1	10.8
Sample No. 2 (8 h)	1.48932	0.448135	0.7	11
Sample No. 3 (16 h)	1.29463	0.5885	0.6	10.3

EXAMPLE 2

This example demonstrates the ability of three different microorganisms to convert the carbohydrate fraction of the hydrolyzate into ethanol and lactic acid. An undiluted hydrolysis liquor was prepared at pH 9.0, at 90°C for 1 hour, with a TiO₂ to lignin-derived compound ration of about 4/1. The hydrolysis liquor was either an acid hydrolyzate of a poplar hardwood or a mixture of softwoods. Three microorganisms *r. Zymomonas mobilis*, *Saccharomyces cerevisiae* D₅A, and *Lactobacillus rhamnosus* were used to evaluate the conversion efficiency of an undiluted hydrolysis liquor to ethanol and lactic acid. The fermentations were carried out in a chemostat culture, the results of which were measured and reported as the percent product yield of a glucose control. The results of these fermentation's are summarized in Table 2.

TABLE 2

Liquor Feedstock Concentration (vol./vol.)	Microorganism	Product	Best Yield (% Glucose Control)
Poplar - hardwood (80%)	<i>Zymomonas mobilis</i>	ethanol	90
QLG mixed softwoods (80%)	<i>Saccharomyces cerevisiae</i>	ethanol	123
QLG mixed softwoods (80%)	<i>Lactobacillus rhamnosus</i>	L(+) Lactic acid	100

A hydrolyzate concentration of 80% (v/v) represents the maximum or full-strength batch-mode fermentation, because the remaining 20% is a 10% (v/v) inoculum and a 10X strength nutrient media. A 30% liquor concentration means that the fermentation has 30% (v/v) hydrolyzate, 20% inoculation media, 2% supplemental sugars and the balance is water.

The detoxification of various hydrolysis liquors is demonstrated in Table 2. Results are shown for the fermentation of both hardwood and softwood liquors. Two

different organisms, *r. Z. mobilis* and *S. cerevisiae*D₅A were used for the fermentation of the detoxified liquors to produce ethanol. The third table entry shows that, for the process of the invention, detoxification also enhances the biological conversion of hydrolyzed sugars to lactic acid using an *L. rhaminosus* organism. The performance of the yeast *S. cerevisiae* D₅A, in converting sugars in the softwood liquor to ethanol was better than the pure sugar control. The enhanced fermentation of the hydrolysis liquor may be due to the buffering effect of other hydrolysis liquor components and a slightly higher initial sugar level in the hydrolysis test sample.

While the present invention has been illustrated and described with reference to particular structures and methods of fabrication, it will be apparent that other changes and modifications can be made therein with the scope of the present invention as defined by the appended claims.

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Claims

1. A process of making a fuel or chemical from a biomass hydrolyzate comprising the steps of:
 - (a) providing a biomass hydrolyzate;
 - (b) adjusting pH of the hydrolyzate;
 - (c) contacting a metal oxide, having an affinity for guaiacyl or syringyl functional groups or both, and the hydrolyzate for a time sufficient to form an adsorption complex;
 - (d) removing the complex wherein a dissolved sugar fraction is provided; and
 - (e) converting the sugar into a fuel or chemical using a microorganism.
2. The process of claim 1 wherein the metal oxide is selected from the group consisting of titanium dioxide, vanadium oxide, and zirconium oxide.
3. The process of claim 1 wherein the adsorbed complex comprises a compound consisting essentially of lignin-derived phenol compounds.
4. The process of claim 1 wherein the pH is adjusted in the range of 6.0 to 9.2.
5. The process of claim 1 further comprising, after adjusting, heating the hydrolyzate to a temperature in the range of 80°C to 100°C.
6. The process of claim 1 wherein the microorganisms are selected from the group consisting of *r. Zymomonas mobilis*, *Saccharomyces cerevisae D₅A*, or *Lactobacillus rhamnosus*.
7. The process of claim 3 wherein the metal oxide comprises titanium oxide, the titanium oxide concentration being twice a phenol concentration of the hydrolyzate.

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8. The process of claim 3 wherein the hydrolyzate is a softwood and the metal oxide concentration is four times a phenol content of the hydrolyzate.
9. The process of claim 4 wherein the dissolved sugar fraction includes less than one mg/mL of lignin-derived compounds.
10. A fermentable medium comprising the undiluted sugar fraction of claim 1.

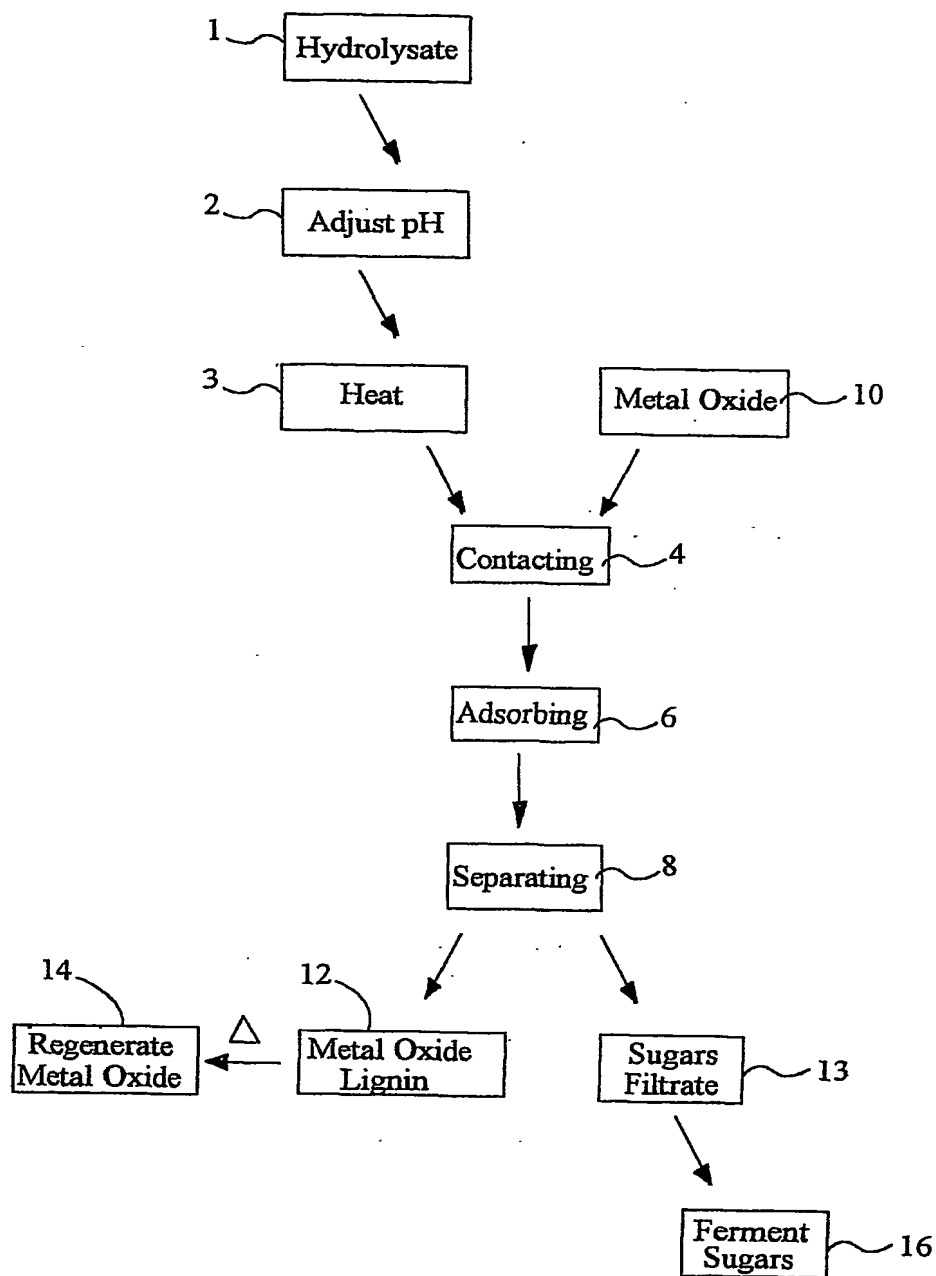
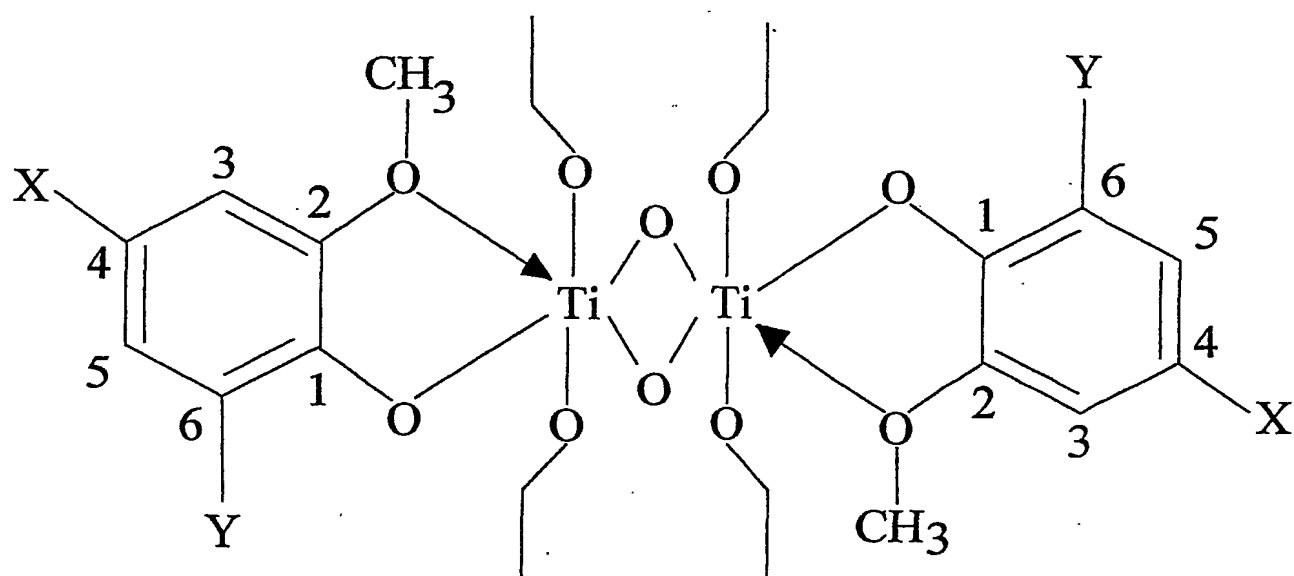


FIG. 1

*FIG. 2*

INTERNATIONAL SEARCH REPORT

Internatid Application No

US 01/11825

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N1/38 C12N1/22 C12P7/56 C12P7/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PEREGO P ET AL: "Acid hemicellulose hydrolysate: Physical treatments and continuous immobilized-cell fermentations." BIOPROCESS ENGINEERING, vol. 10, no. 1, 1994, pages 35-41, XP001016151 ISSN: 0178-515X the whole document	1,3,10
X	US 3 998 732 A (SOLBACH HERMANN ET AL) 21 December 1976 (1976-12-21) the whole document --- -/-	1,3,10

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Lejeune, R



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INTERNATIONAL SEARCH REPORT

International Application No.
US 01/11825

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	<p>MACHADO A E H ET AL: "PHOTOCATALYTIC DEGRADATION OF LIGNIN AND LIGNIN MODELS, USING TITANIUM DIOXIDE: THE ROLE OF THE HYDROXYL RADICAL" CHEMOSPHERE, PERGAMON PRESS, OXFORD, GB, vol. 40, no. 1, 2000, pages 115-124, XP001024191 ISSN: 0045-6535 the whole document</p>	

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International application No

PCT/US 01/11825

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